IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 6525
Richard Daifuku et al.	Examiner: Devesh Khare
Application No.: 10/670,915	Technology Center/Art Unit: 1623
Filed: September 24, 2003	
For: 1,3,5-TRIAZINES FOR TREATMENT OF VIRAL DISEASES	DECLARATION OF DMITRI SERGUEEV UNDER 37 CFR § 1.132
Customer No.: 20350	

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dmitri Sergueev declare as follows:

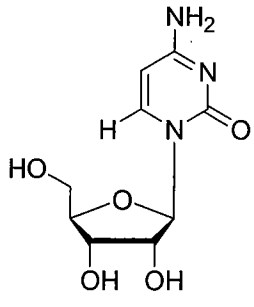
1. I am a research scientist at Sonus Pharmaceuticals. I earned a Ph.D. degree in chemistry from the Novosibirsk Institute of Bioorganic Chemistry in Russia, and a M.S. degree from Novosibirsk State University in Russia. I have worked in the oligonucleotide/nucleoside area for 20 years publishing over 35 peer-reviewed publications and receiving 3 patents. Following my Ph.D., I worked at Duke University as a research scientist where I was awarded the "Armstrong Fellow in Cancer" fellowship. Prior to joining Sonus Pharmaceuticals, I worked at Koronis Pharmaceuticals. My *Curriculum Vitae* is attached as Exhibit 1.

2. The invention is directed to novel non-aromatic triazine nucleoside compounds.

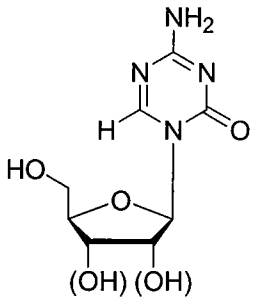
3. I am a named inventor on this patent application. I have read and am familiar with the contents of this patent application. In addition, I have read the Final Office Action mailed May 2, 2007, for the present application. It is my understanding that the pending claims have been rejected because the Examiner believes the claims are obvious over Driscoll *et al.* in combination with Wierenga in view of Meyer *et al.* The Examiner asserts that one of skill in the art, starting from the compounds of Driscoll *et al.*, would arrive at the compounds of the present invention through routine experimentation. I respectfully disagree with the Examiner.

4. This declaration is provided to establish that one skilled in the art would not arrive at the non-aromatic triazine nucleosides of the present invention through routine experimentation starting from the aromatic triazine nucleosides of the cited art.

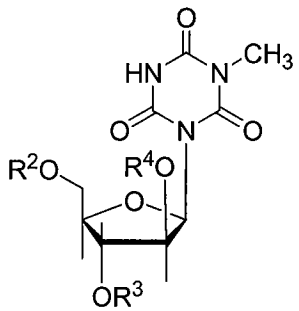
5. The triazines of the present invention are non-aromatic and the triazine compounds of the cited art are aromatic. The nucleosides of Driscoll *et al.* and Wierenga are cytidine analogs. Cytidine (see structure below) is characterized by having a 4-amino-pyrimidin-2-one ring linked to a ribofuranose. The pyrimidine ring has two ring nitrogens, and is *aromatic* as a result of unsaturation at the 3,4- and 5,6-positions, and the 1-aza and 2-carbonyl moieties. The compounds of Driscoll *et al.* and Wierenga are cytidine analogs. Instead of a pyrimidine ring as in cytidine, Driscoll *et al.* and Wierenga use a triazine ring. But like cytidine, the triazines of Driscoll *et al.* are *aromatic* as a result of unsaturation at the 3,4- and 5,6-positions, and the 1-aza and 2-carbonyl that provide the necessary additional conjugation. Likewise, the triazine ring of Wierenga is *aromatic*, despite the lack of any double-bonds in the triazine ring. The Wierenga triazine ring is aromatic because the lone pair of electrons on each ring nitrogen are conjugated as a consequence of being linked via carbonyl carbons at the 2-, 4- and 6-positions.



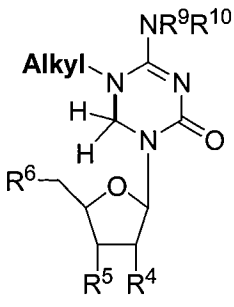
Cytidine
(aromatic)



Driscoll
(aromatic)



Wierenga
(aromatic)

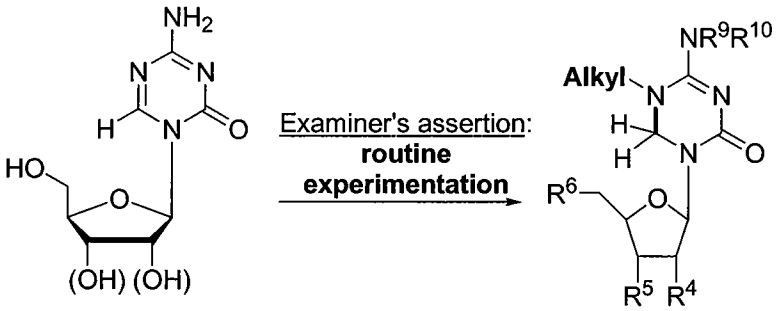


Claim 1
(<u>non</u>-aromatic)

In contrast to the *aromatic* triazines of Driscoll *et al.* and Wierenga, the triazines of the instant invention are *non-aromatic*. The triazines of the instant invention are unsaturated in the 5,6-position with no carbonyl in the 6-position. In fact, the 6-position has two hydrogens. In order for the triazines of the instant claims to be aromatic, there could only be a single hydrogen in the 6-position of the ring. Thus, the conjugation at the 6-position necessary for aromaticity that is present in the compounds of both Driscoll *et al.* and Wierenga, is *absent* in the triazines of the instant claims.

6. Non-aromatic triazines and aromatic triazines, while similar in structure, have vastly different properties. The differences in properties are reflected in inhibition profile, activity, toxicity and mutagenicity, to name a few.

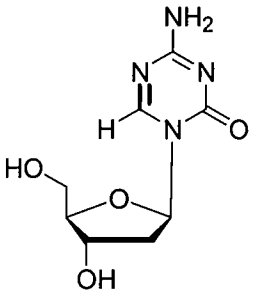
Driscoll *et al.* discloses nucleotides with a 1,3,5-triazine that is *aromatic* as a result of being *fully saturated*, and that is *unsubstituted* at the 5-position. According to the Examiner's assertion, **routine experimentation** with the nucleotides of Driscoll *et al.* would lead one of skill in the art to the *non-aromatic, partially unsaturated* triazines of the instant invention being *substituted* at the 5-position. However, the change from aromatic to non-aromatic triazines leads to significant changes in the properties and behavior of the compounds.



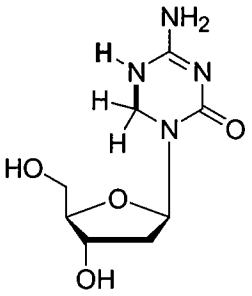
Driscoll
(aromatic)

Claim 1
(non-aromatic)

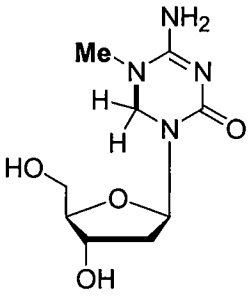
For example, Figure 4 of the instant application shows anti-viral activity of the *aromatic* nucleoside 5-Aza-dC and the *non-aromatic* nucleosides DHAdC and 5-Me-DHAdC (see below).



5-Aza-dC
(aromatic)

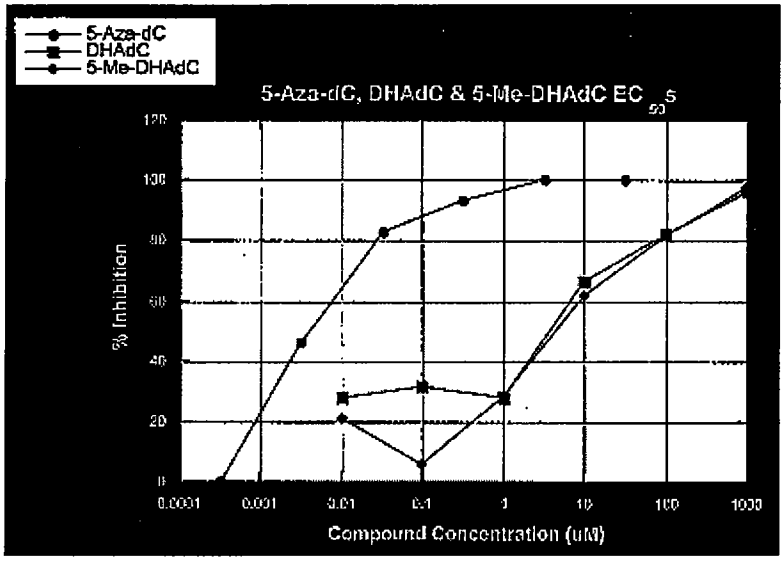


DHAdC
(non-aromatic)



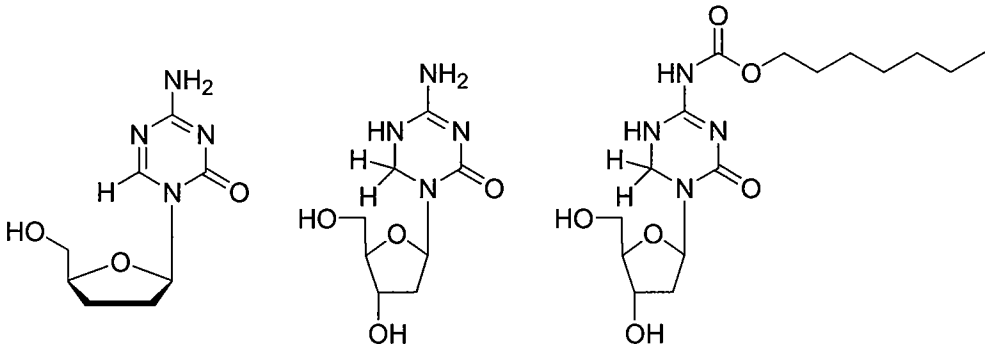
5-Me-DHAdC
(non-aromatic)

Figure 4 shows the inhibition profile for the *aromatic* nucleoside 5-Aza-dC as steadily increasing inhibition up to 80%, above which the inhibition profile levels off and asymptotically approaches 100% inhibition. Despite the small structural differences between the aromatic 5-Aza-dC and the non-aromatic DHAdC and 5-Me-DHAdC, the inhibition profiles for the *non-aromatic* nucleosides DHAdC and 5-Me-DHAdC show a much different profile. The inhibition profiles for the non-aromatic nucleosides remain relatively low (below 40% inhibition) until a critical concentration is reached, at which point the percent inhibition increases steadily to 100%, with no asymptotic approach to 100% inhibition. Thus, despite the seemingly minor differences between the *aromatic* nucleoside 5-Aza-dC and the *non-aromatic* nucleosides DHAdC and 5-Me-DHAdC, the change from an *aromatic* triazine to a *non-aromatic* triazine results in significantly different inhibition profiles.



The Figure 4 inhibition profiles of the *aromatic* 5-Aza-dC and *non-aromatic* DHAdC and 5-Me-DHAdC demonstrate that the aromaticity of the triazine ring plays a significant role in the properties and function of the nucleosides.

7. The effect on aromatic vs. non-aromatic triazine is also exemplified by the data below, showing toxicity levels for non-aromatic triazines KP-1212 and KP-1461 versus the aromatic triazine ddC (dideoxy cytidine).



ddC
(aromatic)

KP-1212
(non-aromatic)

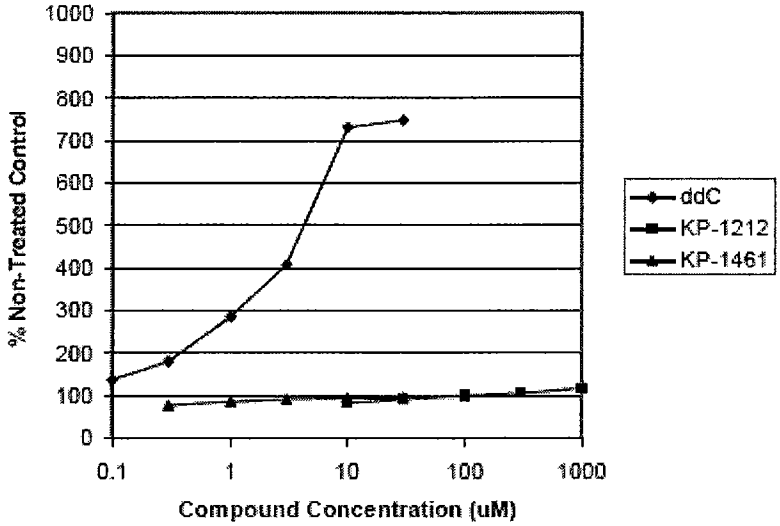
KP-1461
(non-aromatic)

The toxicity levels for the compounds were determined by examining the inhibition of mtDNA synthesis in mammalian cells (Feng et al., 2001), specifically by analyzing lactate and mtDNA production in drug-treated CEM cells.

Lactate and mtDNA production by CEM cells exposed to KP-1212 and KP-1461 were measured. CEM cells were seeded at 3 x 104 cells/well in 96-well plates. Dideoxycytidine

(ddC) was included as a positive control. At the maximum concentrations of ddC, KP-1461, and KP-1212 that were tested, less than 10% inhibition of cell growth was demonstrated in the CEM cytotoxicity assay. After a 72-hour incubation at 37°C, 3 x 10^4 cells per well were transferred to a new 96-well plate. The culture media was replaced with fresh media containing KP-1212, ddC, or KP-1461. The cells were grown and passaged for 9 days. The supernatants were then collected for each drug concentration and lactic acid concentrations were measured with a commercially available diagnostic kit (Sigma, MO) and normalized to 10^6 cells.

The lactic acid concentrations are shown in the figure below as a percentage of the non-treated control. Lactic acid concentration correlates to toxicity, with higher lactic acid concentration equaling higher toxicity levels of the compound. The lactic acid concentration for the non-aromatic KP-1212 and KP-1461 show lactic acid concentration at about the levels for the non-treated control. As the lactic acid concentration correlates to toxicity, this demonstrates that the toxicity for KP-1212 and KP-1461 is about the same as that for the non-treated control.



The lactic acid concentration for the aromatic ddC shows lactic acid concentration as much as 700% higher than that of the non-treated control. As the lactic acid concentration correlates to toxicity, this demonstrates that the toxicity for the aromatic ddC is several times higher than for the non-treated control.

This data further demonstrates that modification of the triazine from aromatic to non-aromatic has a significant impact on the properties of the compound.

8. As one of skill in the art, the aromatic triazines of Wierenga would not motivate me to prepare the non-aromatic triazines of the instant application as the aromaticity of the species results in significantly different results. Furthermore, such non-aromatic triazines would not be arrived at through routine experimentation starting from aromatic triazines.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: Oct. 23, 2007

Dmitri Sergueev, Ph.D.

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RESUME

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Organic/Bioorganic Chemist

OBJECTIVE Seeking a research position in biotech / pharmaceutical industry

SKILLS

- Advanced organic synthesis (heterocycles, carbohydrates, nucleosides, etc.)
- Hands-on experience in drug discovery and development, expanding IP base
- Proficient in essential synthetic and analytical techniques and instrumentation
- Laboratory scale, scale-up, API production under GMP, troubleshooting
- On-line database research for efficient decision-making
- Team or independent work / supervisory skills

EXPERIENCE

Sonus Pharmaceuticals, Inc. Bothell, WA

03/2007- to present RESEARCH SCIENTIST III

- Design and synthesis of anticancer nucleoside analogs
- Development of vitamin conjugates for drug delivery
- State-of the art development in drug screening methodology

Koronis Pharmaceuticals, Inc. Redmond, WA

11/2001-03/2007 SCIENTIST/CHEMIST II

- Design and synthesis of antiviral nucleoside analogs
- Process development and scale-up synthesis
- API production under GMP

Duke University, Department of Chemistry Durham, NC

1/1996-11/2001 RESEARCH ASSOCIATE / SENIOR RESEARCH ASSOCIATE

- Development of new synthetic methods for backbone-modified oligonucleotides
- Study of biochemical properties of novel modified oligonucleotides
- Synthesis of peptide-oligonucleotide-reporter conjugates for antisense applications
- Grant proposals composition; supervision of graduate students

Novosibirsk Institute of Bioorganic Chemistry Novosibirsk, Russia

9/1987-8/1995 JUNIOR RESEARCH SCIENTIST / RESEARCH SCIENTIST

- Methods development for synthesis of novel oligonucleotide-antibiotic conjugates
- Study of DNA cleavage by the oligonucleotide-antibiotic conjugates
- Hybridization studies of the oligonucleotide conjugates and complementary targets
- Discovery of the catalytic DNA cleavage by oligonucleotide-bleomycin tandems
- Supervision of graduate students
- 18 peer-reviewed publications

ADDITIONAL WORK EXPERIENCE

Bayer AG, Central Research and Development Leverkusen, Germany

9/1995-12/1995 FELLOWSHIP POSITION with Dr. Axel Kretschmer

- Synthesis of monomers for the novel peptide nucleic acids

EDUCATION Novosibirsk Institute of Bioorganic Chemistry, Russia

3/1995	**Ph.D.** in Chemistry. Dissertation Title: "Bleomycin Oligonucleotide Derivatives: Synthesis and Effective Site-Specific Cleavage of DNA"
6/1987	Novosibirsk State University, Russia **MS** in Chemistry (minor in Biology)
AWARDS	1987 Novosibirsk State University. Graduation with Honor 1988 Third Prize on The USSR Student Scientific Work Competition 1994-1995 Presidential Scholarship for young talented scientists 2000 "Armstrong Fellow in Cancer" fellowship

SELECTED PUBLICATIONS (out of 39):

(1) Shaw B., Dobrikov M., Sergueev D., Sergueeva Z., Wan J., Wang X., He K., Porter K., Li P., Lin J. Reading, Writing, and Modulating Genetic Information with Boranophosphate Mimics of Nucleotides, DNA, and RNA. *Annals of the New York Academy of Sciences*, **1002**, 12-29 (2004).

(2) Wang X., Dobrikov M., Sergueev D., Shaw B.R. RNase H Activation by Stereoregular Boranophosphate Oligonucleotide. *Nucleosides, Nucleotides & Nucleic Acids*, **22**, 1151-1153 (2003).

(3) Astriab-Fisher A., Ye D., Sergueev D.S., Fisher M.H., Shaw B.R., Juliano R.L. Evaluating the Specificity of Antisense Oligonucleotide Conjugates: A DNA Array Analyses. *J. Biol. Chem.*, **277**, 22980-22984 (2002).

(4) Sergueev D.S., Sergueeva Z.A., Shaw B.R. Synthesis of oligonucleoside boranophosphates via an H-phosphonate method without nucleobase protection. *Nucleosides, Nucleotides and Nucleic Acids*, **20**, 789-795 (2001).

(5) Sergueeva Z.A., Sergueev D.S., Shaw B.R. Borane-amine complexes - versatile reagents in the chemistry of nucleic acids and their analogs. *Nucleosides, Nucleotides and Nucleic Acids*, **20**, 941-945 (2001).

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(7) Sergueeva Z.A., Sergueev D.S., Ribeiro A.A., Summers J.S., Shaw B.R. Individual isomers of dinucleoside boranophosphates as synthons for incorporation into oligonucleotides. Synthesis and configurational assignment. *Helv. Chim. Acta*, **83**, 1377-1391 (2000).

(8) Hughes J., Astriab A., Yoo H., Alahari S., Liang E., Sergueev D., Shaw B.R., Juliano R.L. In vitro transport and delivery of oligonucleotides. In *Methods in Enzymology*, Ed. M.I. Phillips, Academic Press, Orlando FL, Vol. 313 Part A, 342-358 (1999).

(9) Sergueeva Z.A., Sergueev D.S., Shaw B.R. Rapid and selective reduction of amide group by borane-amine complexes in acyl protected nucleosides. *Nucleosides, Nucleotides and Nucleic Acids* **19** (1&2), 275-282 (2000).

(10) Shaw B.R., Sergueev D.S., He K., Porter K.W., Summers J.S., Sergueeva Z.A., Rait V.K., in "*Methods in Enzymology*", Ed. M.I. Phillips, Academic Press, Orlando FL, Vol. 313 Part A, pp.226-257 (1999).

(11) Sergueeva Z.A., Sergueev D.S., Shaw B.R. Synthesis of dithymidine boranophosphates via stereospecific boronation of H-phosphonate diesters and assignment of their configuration. *Tetrahedron Letters* **40**, 2041-2044 (1999).

(12) Sergueev D.S., Shaw B.R. H-Phosphonate approach for solid-phase synthesis of oligonucleoside boranophosphates and their characterization. *J. Am. Chem. Soc.*, **120**, 9417-9427 (1998).

(13) Sergueev D., Hasan A., Ramaswamy M., Shaw B. R. Boranophosphate oligonucleotides: New synthetic approaches. *Nucleosides & Nucleotides*, **16**, 1533-1538 (1997).

(14) Sergeev D.S., Zarytova V. F. Interaction of bleomycin and its oligonucleotide derivatives with nucleic acids. *Russ. Chem. Rev.*, **65**, 355-378 (1996).

(15) Sergeyev D.S., Godovikova T.S., Zarytova V.F. Site-specific catalytic cleavage of the DNA-target by oligonucleotide bearing bleomycin A5. *Nucleic Acids Res.*, **21**, 4400-4406 (1995).

(16) Zarytova V.F., Sergeyev D.S., Godovikova T.S. Synthesis of Bleomycin A5 Oligonucleotide Derivatives and Site-Specific Cleavage of the DNA Target. *Bioconjugate Chem.*, **4**, 189-193 (1993).

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PATENTS

(1) Shaw B.R., Porter K.W., Sergueev D. Synthesis of oligonucleotides with boranophosphate linkages. US patent 5,859,231 (1999).

(2) Shaw B.R., Porter K.W., Sergueev D. Method of nucleic acid sequencing. US patent 6,376,178 (2002).

(3) Shaw B.R., Porter K.W., Sergueev D. Method of nucleic acid sequencing. US patent 6,808,897 (2004).

(4) Daifuku R., Gall A., Sergueev D. 1,3,5-Triazines for treatment of viral diseases. Provisional patent application (2003).

(5) Daifuku R., Gall A., Sergueev D. Mutagenic heterocycles. Provisional patent application (2003).

(6) Daifuku R., Gall A., Sergueev D. Prodrug of heteroaryl compounds. Provisional patent application (2004).

PRESENTATIONS AT INTERNATIONAL SCIENTIFIC CONFERENCES

1. Sergueev D., Shaw B. R. Synthesis and anti-HIV Activity of 5-Aza-2'-deoxy-5,6-dihydro-5-methylcytidine. *Gordon Research Conference on Purines, Pyrimidines and Related Substances*, Newport, RI, June 30-July 4, 2003.
2. Sergueev D., German V., Shaw B. R. Synthesis of Oligonucleotides with Single Boranophosphate Substitution. *Gordon Research Conference on Purines, Pyrimidines and Related Substances*, Newport, RI, June 28-July 2, 2001.
3. Sergueev D.S., Sergueeva Z.A., Shaw B.R. Synthesis of Oligonucleoside Boranophosphates via an H-Phosphonate Method without Nucleobase Protection. *XIV International Roundtable "Nucleosides, Nucleotides, and Their Biological Applications"*, San Francisco, CA, September 10-14, 2000.
4. Sergueev D.S., Sergueeva Z.A., Summers J.S., Shaw B.R. Synthesis of Dinucleoside Boranophosphates via Stereospecific Boronation of H-phosphonate Diesters and Assignment of their Configuration. *Millennium Conference on Nucleic Acid Therapeutics*, Clearwater Beach, FL, January 8-11, 2000.
5. Sergueev D., Shaw B. R. Boranophosphate Oligonucleotides: New Synthetic Approaches. *Gordon Research Conference on Purines, Pyrimidines and Related Substances*, Newport, RI, June 30-July 4, 1997.
6. Sergueev D., Hasan A., Ramaswamy M., Shaw B.R. Boranophosphate Oligonucleotides: New Synthetic Approaches. *XII International Round Table "Making Drugs Out of Nucleosides and Oligonucleotides"*, LaJolla, CA, September 15-19, 1996.